

U.S. Serial No. 09/667,569  
Attorney Docket: BGI-141CP

Group Art Unit: 1652  
Examiner: D. Steadman

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1. (Original) A method of producing a panto-compound comprising culturing a microorganism which overexpresses at least one *Bacillus* pantothenate biosynthetic enzyme under conditions such that the panto-compound is produced.

Claim 2. (Original) The method of claim 1, wherein the microorganism overexpresses at least one *Bacillus subtilis* pantothenate biosynthetic enzyme.

Claims 3-6. (Canceled)

Claim 7. (Original) A method of producing a panto-compound comprising culturing a ketopantoate reductase-overexpressing (KPAR-O) microorganism under conditions such that the panto-compound is produced.

Claims 8-11. (Canceled)

Claim 12. (Previously Presented) The method of claim 7, wherein the KPAR-O microorganism further overexpresses at least one pantothenate biosynthetic enzyme in addition to overexpressing ketopantoate reductase.

Claim 13. (Canceled)

Claim 14. (Withdrawn) A method of producing pantothenate in a manner independent of precursor feed comprising culturing an aspartate- $\alpha$ -decarboxylase-overexpressing (A $\alpha$ D-O) microorganism having a deregulated isoleucine-valine (*i/v*) pathway under conditions such that pantothenate is produced.

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Claim 15. (Withdrawn) A method of producing at least 2 g/L pantothenate in a manner independent of aspartate or  $\beta$ -alanine feed comprising culturing an aspartate- $\alpha$ -decarboxylase-overexpressing (A $\alpha$ D-O) microorganism under conditions such that pantothenate is produced.

Claim 16. (Withdrawn) A method of producing at least 2 g/L pantothenate in a manner independent of valine or  $\alpha$ -ketoisovalerate feed comprising culturing a microorganism having a deregulated isoleucine-valine (*ilv*) biosynthetic pathway under conditions such that pantothenate is produced.

Claim 17. (Withdrawn) A method of producing at least 30 g/L pantothenate in a manner independent of aspartate or  $\beta$ -alanine feed comprising culturing an aspartate- $\alpha$ -decarboxylase-overexpressing (A $\alpha$ D-O) microorganism under conditions such that pantothenate is produced.

Claim 18. (Withdrawn) A method of producing at least 30 g/L pantothenate in a manner independent of valine or  $\alpha$ -ketoisovalerate feed comprising culturing a microorganism having a deregulated isoleucine-valine (*ilv*) biosynthetic pathway under conditions such that pantothenate is produced.

 Claim 19. (Currently Amended) A  $\beta$ -alanine independent high yield production method for producing pantothenate comprising culturing a manipulated microorganism under conditions such that pantothenate is produced at a significantly high yield, as compared to an un-manipulated or wild-type microorganism.

Claim 20. (Withdrawn) The method of claim 14 or 19, wherein the microorganism overexpresses acetohydroxyacid synthetase or is transformed with a vector comprising an *ilvBN* nucleic acid sequence or an *alsS* sequence.

Claim 21. (Withdrawn) The method of claim 14 or 19, wherein the microorganism overexpresses acetohydroxyacid isomeroreductase or is transformed with a vector comprising an *ilvC* nucleic acid sequence.

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Claim 22. (Withdrawn) The method of claim 14 or 19, wherein the microorganism overexpresses dihydroxyacid dehydratase or is transformed with a vector comprising an *ilvD* nucleic acid sequence.

Claim 23. (Withdrawn) The method of claim 19, wherein the microorganism overexpresses aspartate- $\alpha$ -decarboxylase or is transformed with a vector comprising a *panD* nucleic acid sequence.

Claim 24. (Previously Presented) The method of claim 14 or 19, wherein the microorganism further has a deregulated pantothenate biosynthetic pathway.

Claim 25. (Withdrawn) The method of claim 14 or 19, wherein the microorganism further has at least one mutant gene selected from the group consisting of a mutant *avlA* gene, a mutant *ilvE* gene, a mutant *ansB* gene and a mutant *alsD* gene.

Claim 26. (Original) The method of claim 24, wherein the microorganism overexpresses any of ketopantoate hydroxymethyltransferase, ketopantoate reductase, pantothenate synthetase and aspartate- $\alpha$ -decarboxylase.

Claim 27. (Previously Presented) The method of claim 24, wherein the microorganism is transformed with a vector comprising a *panBCD* nucleic acid sequence or a vector comprising a *panE1* nucleic acid sequence.

Claim 28. (Previously Presented) The method of claim 14 or 19, wherein pantothenate is produced at a level selected from the group consisting of a level greater than 10g/L, a level greater than 20g/L and a level greater than 40g/L.

Claim 29. (Withdrawn) The method of claim 20, wherein the microorganism overexpresses acetohydroxyacid synthetase derived from *Bacillus* or is transformed with a vector comprising an *ilvBN* nucleic acid sequence or an *alsS* nucleic acid sequence derived from *Bacillus*.

Claim 30. (Withdrawn) The method of claim 21, wherein the microorganism overexpresses acetohydroxyacid isomeroreductase derived from *Bacillus* or is transformed with a vector comprising an *ilvC* nucleic acid sequence derived from *Bacillus*.

Claim 31. (Withdrawn) The method of claim 22, wherein the microorganism overexpresses dihydroxyacid dehydratase derived from *Bacillus* or is transformed with a vector comprising an *ilvD* nucleic acid sequence derived from *Bacillus*.

Claim 32. (Withdrawn) The method of claim 23, wherein the microorganism overexpresses aspartate- $\alpha$ -decarboxylase derived from *Bacillus* or is transformed with a vector comprising a *panD* nucleic acid sequence derived from *Bacillus*.

Claim 33. (Previously Presented) The method of claim 24 or 26, wherein the microorganism overexpresses any of ketopantoate hydroxymethyltransferase, ketopantoate reductase, pantothenate synthetase and aspartate- $\alpha$ -decarboxylase derived from *Bacillus*.

Claim 34. (Original) The method of claim 27, wherein the vector comprises a *panBCD* nucleic acid sequence or a *panE1* nucleic acid sequence derived from *Bacillus*.

Claim 35. (Canceled)

Claim 36. (Withdrawn) A method of producing  $\beta$ -alanine comprising culturing an aspartate- $\alpha$ -decarboxylase-overexpressing (A $\alpha$ D-O) microorganism under conditions such that  $\beta$ -alanine is produced.

Claim 37. (Withdrawn) The method of claim 36, wherein the A $\alpha$ D-O microorganism has a mutation in a nucleic acid sequence encoding a pantothenate biosynthetic enzyme selected from the group consisting of ketopantoate hydroxymethyltransferase, ketopantoate reductase and pantothenate synthetase.

Claim 38. (Canceled)

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Claim 39. (Withdrawn) A method for enhancing production of a panto-compound comprising culturing a mutant microorganism having a mutant *coaX* gene under conditions such that the panto-compound production is enhanced.

Claim 40. (Withdrawn) The method of claim 39, wherein said recombinant microorganism has a mutant *coaA* gene.

Claim 41. (Withdrawn) A method of producing a panto-compound comprising culturing a pantothenate kinase mutant microorganism under conditions such that the panto-compound is produced at a significantly high yield.

Claim 42. (Withdrawn) The method of claim 41, wherein said mutant microorganism has a mutant *coaA* gene.

Claim 43. (Withdrawn) The method of claim 41, wherein said mutant microorganism has a mutant *coaX* gene.

Claim 44. (Withdrawn) The method of claim 41, where said mutant microorganism has a mutant *coaA* and *coaX* gene.

Claim 45. (Canceled)

Claim 46. (Withdrawn) The method of claim 39 or 41, wherein said panto-compound is pantothenate.

Claim 47. (Withdrawn) The method of claim 39 or 41, wherein said panto-compound is produced at a level selected from the group consisting of a level greater than 10g/L, a level greater than 20g/L and a level greater than 40g/L.

Claim 48. (Withdrawn) The method of claim 39 or 41, wherein said recombinant microorganism further has a deregulated pantothenate biosynthetic pathway or further has a deregulated isoleucine-valine (*ilv*) biosynthetic pathway.

Claim 49. (Previously Presented) The method of claim 39 or 41, wherein said recombinant microorganism further overexpresses *panD* and *panE*.

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Claim 50. (Withdrawn) The method of claim 39 or 41, wherein said recombinant microorganism further has at least one mutant gene selected from the group consisting of a mutant *avtA* gene, a mutant *ilvE* gene, a mutant *ansB* gene and a mutant *alsD* gene.

Claim 51. (Previously Presented) A method for enhancing production of a panto-compound comprising culturing a microorganism that has a deregulated pantothenate biosynthetic pathway and that also has a mutation that results in reduced pantothenate kinase activity under conditions such that the panto-compound production is enhanced.

Claims 52-110. (Canceled)

Claim 111. (New) A method for producing a panto-compound comprising:

(a) transforming a microorganism with a vector to produce a recombinant microorganism, wherein said vector comprises a *panE* gene with 95% or greater amino acid identity to SEQ ID No:30, operably linked to a constitutively active promoter set forth in SEQ ID NO:40, and further operably linked to a regulatory sequence comprising an artificial ribosome binding site (RBS), set forth in SEQ ID No:50;

(b) growing said recombinant microorganism in an appropriate culture medium and under conditions suitable for the production of said panto-compound; and

(c) recovering said panto-compound from said culture medium.

Claim 112. (New) A method for producing a panto-compound comprising:

(a) transforming a microorganism with a vector to produce a recombinant microorganism, wherein said vector comprises a *panE* gene with 95% or greater amino acid identity to SEQ ID No:30, operably linked to a constitutively active promoter set forth in SEQ ID NO:40, and further operably linked to a regulatory sequence comprising an artificial ribosome binding site (RBS), set forth in SEQ ID No:50;

(b) further transforming said recombinant microorganism with a vector comprising a *panBCD* operon with 95% or greater amino acid identity to SEQ ID No: 24, 26, and 28, operably linked to a constitutively active promoter set forth in SEQ ID

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NO:40, and further operably linked to a regulatory sequence comprising an artificial ribosome binding site (RBS), set forth in SEQ ID No:50;

(c) growing said recombinant microorganism in an appropriate culture medium and under conditions suitable for the production of said panto-compound; and

(d) recovering said panto-compound from said culture medium.

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Claim 113. (New) The method of claim 112, wherein said recombinant microorganism is further transformed with a plasmid set forth in SEQ ID No: 78. (to overexpress panD)

Claim 114. (New) The method of claim 113, wherein said recombinant microorganism is further transformed with a plasmid set forth in SEQ ID No: 82. (to deregulate ilvBNC)

Claim 115. (New) The method of claim 114, wherein said recombinant microorganism is modified to deregulate the *Bacillus subtilis* *ilvD* gene.

Claim 116. (New) The method of any one of claims 111-115, wherein said appropriate culture medium contains valine.